Report No.

Title: Static Acute Toxicity of to *Daphnia magna* Straus

Study No.:

Test Article:

Study Director:
Authors:

Sponsor:

Testing Facility:

Sponsor Representative:

Study Completion Date: January 9, 2001

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ABSTRACT

A 48-h static acute toxicity test of to *Daphnia magna* Straus was conducted by the

Nominal test concentrations were 0, 10, 18, 32, 56, and 100 mg/L. Analytical confirmation during this test was done by a validated ICP-OEC method and is reported within.

This study was conducted in accordance with (except as noted) the Organisation for Economic Co-operation and Development (OECD) 202, *Daphnia* sp., Acute Immobilisation Test and Reproduction Test, 1984 and EU C.1. 1992.

The test article readily dissolved in the water at all test concentrations forming a clear test solution without observable films or precipitates. The analytically determined levels at 0 hours were between 90 and 102% of nominal concentrations. At 48 hours the percent remaining of originally measured levels ranged from 93 – 99%. These results confirm test article stability and homogeneity through the study period.

No mortality was observed in the controls or 18 mg/L test solutions at 24 and 48 hours. The single mortality occurring at 10 mg/L was unlikely to be test article related. All daphnids were dead at 100 mg/L by the end of the test and substantial mortalities occurred at intermediate concentrations. The calculated LC50 for 24 hours was 63 mg/L (95% Confidence interval 52 – 78 mg/L)and was 34 mg/L for 48 hours (95% Confidence interval 30 – 39 mg/L). The No Observable Adverse Effect Concentration was 18 mg/L.

Based upon the results of this definitive test, is slightly toxic to *Daphnia magna*.

GLP COMPLIANCE STATEMENT

Following is the GLP Compliance Statement for the report, "Static Acute Toxicity of to *Daphnia magna* Straus" prepared by

study director for the above study confirms that this study with the exception of:

- computerized statistical programs not validated under GLPs

was conducted in compliance with the Organization for Economic Co-operation and Development (OECD) Good Laboratory Practices (1997).

There were no circumstances known that would negatively impact or bias results of this study.

Jan. 9, 2001
Date

Aquatic Toxicologist

Study Director

QUALITY ASSURANCE STATEMENT

Title: Static Acute Toxicity of to *Daphnia magna* Straus

Study Number:

This study has been audited by the Quality Assurance Unit according to approved Standard Operating Procedures to assure that the raw data are accurately reflected within this final report. The following are the inspection dates and the dates that the inspection findings were reported.

Dates of Inspection	Phase Inspected	Findings Reported to Study Director	Findings Reported to Management
16-17 May 00	Draft Protocol Review	18 May 00	19 May 00
01 Aug 00	Loading and Randomization	01 Aug 00	03 Aug 00
03 Aug 00	48-h Test System Observation	03 Aug 00	04 Aug 00
1, 4-6 Dec 00	Draft Final Report, Associated Raw Data Review	08 Dec 00	04 Jan 01

08 Jan 01
Date

*Manager, Quality Assurance

APPROVAL SIGNATURES

This report consists of pages 1 through 26 including Tables 1 through 6 and Appendices A and B.

Jan. 9, 2001
Date

Aquatic Toxicologist

09-Jan-01
Date

Team Leader, Chemistry & Environmental Sciences Group

STUDY INFORMATION

Study Initiation Date:	July 25, 2000
Experimental Start Date:	August 1, 2000
Experimental Termination Date:	August 3, 2000
Study Completion Date:	January 9, 2001

Study Personnel:

INTRODUCTION

A 48-h static acute toxicity test of to the freshwater daphnid, *Daphnia magna* Straus was conducted by the

This study design incorporated most elements of 'Organisation for Economic Co-operation and Development (OECD) 202, *Daphnia* sp., Acute Immobilisation Test and Reproduction Test, 1984[1] and EU C.1., 1992[2]'. An exception to these guidelines was that it did not include any reproductive aspects as it was planned as an acute test only. *Daphnia magna* represent an important group of warm water invertebrates representing an important link in the aquatic food chain.

MATERIALS AND METHODS

Test Article Specifications

The test article was submitted for GLP characterization prior to conduct of this study. Results indicate that this lot may be considered representative of this product. The following specifications pertain to the batch used in this study:

•Identity:	
• CAS #	Unassigned
•Batch Number:	0000439367
•Physical Description:	Colorless liquid
•Source:	
•Stability:	Stable per MSDS
•Solubility:	Soluble in water at test concentrations
•Purity:	Material was tested as is, assumed to be pure as supplied & representative of product
•Expiration date:	December 17, 2000
•Storage Conditions:	Room temperature
•Handling Precautions:	See MSDS
•Characterization:	GLP –
•Archive requirements:	None

Test Article Analytical Methods

Test solutions were analyzed at 0 and 48 hours by ICP-OES. Test solutions were analyzed directly after introduction of an internal standard (Ge) with a Perkin Elmer Optima 3100XL ICP-OES monitoring for silicon at 251.611 and 288.158 nm. The method is not specific for the test article so appropriate controls and matrix blanks were also run. Reported values were corrected for matrix blanks and converted to test article concentrations by conversion from elemental silicon

as measured by the instrument. Freshly spiked solutions were analyzed for each run to demonstrate precision of method. Details of method may be found in Appendix A.

Test Article Preparation

The test article test solutions were prepared by direct addition of the test article to each test solution. Test article was weighed into tared glass weighing caps which were added to a one-liter volumetric flask partially filled with well water. Actual test article weights used to generate 0, 10, 18, 32, 56, and 100 mg/L were 0, 0.010, 0.018, 0.032, 0.056, and 0.100 g. An empty glass weighing cap was added to the control flask containing unadulterated well water. All solutions were mixed by repeated inversions until test article dispersed and brought to appropriate volume with well water. Test solutions were then distributed into duplicate labeled test vessels. Daphnia neonates were introduced to the test vessels approximately one hour after test solution preparation.

Test Organism and Source

The test organism, *Daphnia magna* Straus is a common test invertebrate used for static acute toxicity testing [2]. The criteria for selection of this testing species is based on its ecological importance, availability, ease of culturing and past success in other toxicity studies. In-house cultures were raised from an initial stock culture received from Aquatic BioSystems Inc., Fort Collins, Colorado, December 1997.

Organism Culture Conditions

The laboratory daphnid cultures are incubated at 20 ± 2 °C, with continuous aeration, under cool white fluorescent lighting with a photoperiod of 16-h light/8-h dark and a photo-intensity of 195 ± 32 foot candles. Daphnia cultures were transferred twice weekly to new media and fed a diet of *Ankistrodesmus convolutus* Corda and Yeast, Cerophyl®, and Trout Chow (YCT) three to four times a week. No contaminants which might effect the outcome of this study are known to be present in the organism food. The day prior to test article exposure neonates were separated from the adult daphnia. Neonates used to start the test were collected from those produced within the next 24-h. Adult daphnia cultures greater than 12-days old, free of ephippia, and having less than 20% mortality during the previous 48-h were used as a source of neonates.

Organism Culture and Test Water

The water was obtained from the water well at 1809 Salzburg Rd., Bay Co., MI. The water was pumped from the well and transported to the laboratory where it was adjusted to 20 ± 2°C and aerated before use. Daphnid water was monitored weekly for pH, alkalinity, conductivity and hardness; and once a year for selected inorganics, total dissolved solids, total suspended solids, total organic carbon, and selected organic compounds. Results of weekly water quality monitoring are

included in Table 1. No contaminants were known to be present at a concentration likely to adversely affect the outcome of this study.

Test Vessels

Test vessels were 250 mL glass beakers containing 200 mL of test solution or laboratory well water. Each test vessel was covered with a watch glass to reduce evaporation. Test vessels were labeled with the study number, test article concentration, replicate number, initials of study director or laboratory personnel and the exposure start date.

Loading and Randomization

Twenty daphnia were exposed to each concentration with ten daphnia per replicate (total of 120 organisms). The test organisms (test system) were exposed in the prepared test vessels after the test solution water quality measurements (temperature, pH, and dissolved oxygen) were completed. Approximately 20% (2 of 10) of the daphnia were added into each beaker beginning with the well water controls and continuing with the beakers containing test substance. This procedure was repeated until the appropriate number of daphnia per replicate was obtained. Once the correct count for each vessel was verified, the beakers were randomly placed in the test incubator. Test vessels were assigned a random placement in the testing incubator using a random number generation program[3].

Test System Observations

Daphnia were observed at 0, 24, and 48 hours to assess mortality and morbidity. Daphnia were considered dead if they exhibited no heart beat or response to gentle prodding. Organisms substantially less active than controls or entrapped were recorded.

Water Quality Measurements

Dissolved oxygen, pH, and temperature were recorded at 0 and 48 hours. Dissolved oxygen was determined using a Yellow Springs Instrument (YSI), model 95 dissolved oxygen meter. An Orion, model 610 pH meter was used to determine pH. Temperature of test solutions was measured using a calibrated mercury thermometer. Incubator temperature was monitored continuously using a Dataplex 32 system designed to alarm if temperatures were out of the designated range (18 - 22°C).

RESULTS

Test Conditions

Test conditions were similar to the culturing conditions except the organisms were not fed during the test and the test vessels were not aerated. Test vessel temperatures ranged from 20.5 – 20.7 °C at 0-h and from 20.2 – 20.9 °C at 48-h. Test vessel temperature data are provided in Table 2. Test vessel temperature was

within the specified range. Incubator temperature during the test period was continuously monitored using the Dataplex System and a thermocouple probe. Recorded Dataplex values were 19 or 20 °C during the study period.

Initial and final test solution pH values are provided in Table 3. Measured pH values ranged from 8.14 - 8.64 at 0-h and from 8.09 - 8.31 at 48-hours. pH values varied with test article concentration but were not thought to influence test results.

Dissolved oxygen concentrations were measured on the test solutions at 0-h and 48-h. The dissolved oxygen (D.O.) values ranged from 8.81 - 8.96 mg/L at 0-h and from 8.52 - 8.65 mg/L at 48-h. Dissolved oxygen levels were within the required 60 to 105% saturation range throughout the 48-h test period. Dissolved oxygen values are shown in Table 4.

GE Cool White and Mod-U-Line E fluorescent lighting with a photoperiod of 16-h light/8-h dark and a photo-intensity of 192 - 205 foot candles was provided throughout the study. Test incubator light readings are provided in Table 5.

Test Article Measurements

Complete results of the test article measurements are provided in Appendix A. The average concentration of the duplicate test vessels measured during the study after correcting for the matrix blanks were:

Test Concentration	*0 Hours mg/L*	*48 Hours mg/L*	*% Remaining after 48 Hours*
0 mg/L	0	1.74	NA
10 mg/L	10.1	9.50	94
18 mg/L	16.2	16.0	99
32mg/L	32.5	30.5	94
56mg/L	56.5	53.8	95
100 mg/L	102	94.7	93
NA = Not applicable			

The measured levels were consistent with the expected nominal concentrations based on known quantity added to test solutions. The amount remaining after 48 hours fell within the range of acceptability for test solution stability (92-104% versus acceptable range of 80 – 120%). These data are consistent with a stable homogenous solution of test article in the well water.

Test System Observations

The test system organisms were observed and response to the test article was recorded at 0-h, 24-h and 48-h. Results are shown in Table 6. Observations were made daily within 1-h of the 0-h, 24-h and 48-h test periods. Dead daphnids were not removed. All organisms appeared healthy and normal at 0-h. At 24-h no effects were observed at 0, 10 and 18 mg/L and there was a single mortality at 32 mg/L. There were substantial mortalities at the highest concentrations. At 48-h,

complete mortalities were observed at the 100 mg/L concentration and 19 were dead and the rest normal at 56 mg/L. Nine had succumbed at 32 mg/L, but the others appeared unaffected. There was a single mortality at 10 mg/L, which was deemed non-test article related as there were no mortalities or untoward effects at 18 mg/L. The test was deemed acceptable since no mortalities or immobilization occurred in the controls.

Calculations and Statistical Analysis

Statistical analyses by probit analysis (SAS version 8) was performed to calculate the 24 and 48 hour median lethal concentrations (Appendix B). Data from 48 hours at 10 mg/L were not included in analysis as the single mortality was considered not dose related. The calculated median lethal concentrations were 63 mg/L (95% confidence level 52 – 78 mg/L) at 24 hours and 34 mg/L (95% confidence level 30 – 39 mg/L) at 48 hours. The no observable adverse effect concentration was 18 mg/L.

Conclusions

is slightly toxic to *Daphnia magna* upon acute exposure.

Deviations to the Protocol

The following protocol deviation was noted:

- Total dissolved solids were not determined for well water during last annual analysis.
- Incubator temperature was not recorded at 0 Hours

Neither deviation is expected to affect outcome of study.

ARCHIVE

The original protocol, amendments, raw data, and final report related to this study are maintained in

ACKNOWLEDGEMENTS

The authors wish to thank for timely statistical analysis.

REFERENCES

1. Organisation for Economic Co-operation and Development (OECD) 202, Daphnia sp., Acute Immobilisation Test and Reproduction Test. 1984. Paris.
2. Official Journal of the European Communities. L 383 A. Volume 35. ISSN 0378-6978. December 29, 1992. Part C: Methods for the determination of Ecotoxicity. C.2. Acute Toxicity for Daphnia.

3. Microsoft® EXCEL for Windows 95, Version 7.0a. Analysis ToolPak - Random Number Generation. Copyright® 1985-1996.

TABLE 1

Table1: Well Water Quality Data During Daphnia Culture & Test Period

Measurement Date	Hardness mg/L as $CaCO_3$	Alkalinity mg/L as $CaCO_3$	pH	Conductivity µmho/cm
6/30/00	96	170	7.3	565
7/7/00	94	163	7.3	555
7/13/00	92	162	7.5	552
7/21/00	101	174	7.2	566
7/28/00	124	197	7.2	605
8/3/00	106	182	7.2	585

TABLE 2

Table 2: Temperature data

Concentration	Test Vessel #	Temperature °C	
		0-h	48-h
Control 0 mg/L	1	20.5	20.4
	2	20.5	20.2
10 mg/L	3	20.5	20.4
	4	20.5	20.5
18 mg/L	5	20.6	20.4
	6	20.6	20.7
32 mg/L	7	20.6	20.8
	8	20.6	20.9
56 mg/L	9	20.6	20.4
	10	20.6	20.5
100 mg/L	11	20.7	20.8
	12	20.7	20.3

TABLE 3

Table 3: pH data

Concentration	Test Vessel #	PH	
		Test Vessel 0-h	Test Vessel 48-h
Control 0 mg/L	1	8.14	8.09
	2	8.14	8.08
10 mg/L	3	8.18	8.22
	4	8.22	8.26
18 mg/L	5	8.27	8.25
	6	8.29	8.28
32 mg/L	7	8.35	8.28
	8	8.38	8.28
56 mg/L	9	8.47	8.31
	10	8.50	8.29
100 mg/L	11	8.60	8.31
	12	8.64	8.31

TABLE 4

Table 4: Dissolved oxygen data

Concentration	Test Vessel #	Dissolved Oxygen mg/L	
		0-h	48-h
Control 0 mg/L	1	8.82	8.52
	2	8.86	8.62
10 mg/L	3	8.83	8.59
	4	8.81	8.60
18 mg/L	5	8.84	8.59
	6	8.84	8.59
32 mg/L	7	8.86	8.56
	8	8.96	8.65
56 mg/L	9	8.93	8.64
	10	8.89	8.62
100 mg/L	11	8.92	8.65
	12	8.87	8.63

TABLE 5

Table 5: Light readings

Location I.D.	Light Readings (foot-candles)		
	0-h	24-h	48-h
1	204	203	205
2	195	201	198
3	194	188	195
4	192	186	196

TABLE 6

Table 6: Test system observations

Concentration mg/L	Test Vessel #	Observations*		
		0-h	24-h	48-h
Control 0 mg/L	1	10N	10N	10N
	2	10N	10N	10N
10 mg/L	3	10N	10N	9N:1D
	4	10N	10N	10N
18 mg/L	5	10N	10N	10N
	6	10N	10N	10N
32 mg/L	7	10N	10N	7N:3D
	8	10N	9N:1D	4N: 6D
56 mg/L	9	10N	7N:3D	10D
	10	10N	2N:8D	1N: 9D
100 mg/L	11	10N	1N:1M:8D	10D
	12	10N	1N:2M:7D	10D

* Observations are coded as: N = Normal, D = Dead, M = Moribund

Appendix A

To:

From:

Re: Analytical Verification of Concentrations in Daphnia Test Water during Conduct of

Development and validation of the ICP-OES method used to measure test concentrations of in aquatic medium were performed as a non-regulated study, which is documented in a separate esearch report.[1] A summary of the ICP method used during is included in this memo. For more detail, refer to the full report referenced above.

Summary of

Five product loadings of n well water were prepared along with controls (well water with Daphnia but no product). Aliquots for the 0-hour measurements were drawn in duplicate directly from the test solution preparation vessels. Single aliquots were drawn from the duplicate test vessels for the 48-hour measurements after removal of the Daphnia. All test solutions, including controls and spikes, were analyzed in triplicate. The average concentrations of measured in the 0- and 48-hour test solutions are shown below along with the percent product remaining in the test vessels at termination of the study. Individual concentration measurements as well as averages for the controls, test solutions, and QC samples from time = 0 and time = 48 hours are included in Tables IV and V, respectively, at the end of this report. The apparent product concentration measured in the test controls at 48 hours is most likely due to Si contamination inadvertently introduced during conduct of the study.

	(mg/L) Measured during Daphnia Study (matrix blank-corrected results)		
Loading Level (mg/L)	**Time = 0 hrs** Aver., Duplicate Samples	**Time = 48 hrs** Aver., Duplicate Vessels	**% Remaining after 48 Hours**
0	-0.650	1.74	NA
10	10.1	9.50	94%
18	16.2	16.0	99%
32	32.5	30.5	94%
56	56.5	53.8	95%
100	102	94.7	93%

Table I NA = not applicable

Materials and Apparatus

(lot #0000439367,
Germanium (Spex, 10,000 microgram/mL in water, lot #J6-137GE)
aerated well water (from DC3 laboratory #207)
125-mL polypropylene bottles (Nalgene®)
glass volumetric pipettes, 50-, 25-, and 10-mL (Fisher Scientific, Class A, borosilicate)
polypropylene volumetric flasks, 50- and 100-mL (Nalgene®, Class B)
polypropylene centrifuge tubes, 15-mL and 50-mL (Fisher Scientific)
Rainin® electronic digital pipette (serial #050509)
100 microliter Unimetrics glass syringe
Mettler® analytical balance (serial #1114513136)

Appendix A (Cont.)

Calibration Standard and Quality Control Stock Solution Preparation
Fresh calibration standard and quality control (QC) stock solutions were prepared each day for the 0- and 48-hour ICP analyses. For each stock, approximately 0.1 grams of test article were weighed into 125-mL Nalgene bottles followed by approximately 135 grams of aerated well water.

Fresh calibration standards were prepared each day of analysis by dilution from the stock solution described above. Target concentrations for the calibration standards were 1, 5, 10, 25, 50, 100, and 150 mg/L test article in well water. A Rainin electronic digital pipette was used to transfer aliquots of the stock solution to 50-mL or 100-mL volumetric flasks, which were weighed using the analytical balance described previously. The aliquots were diluted to the mark with well water in the 50-mL volumetric flasks. The resulting test article concentration was calculated using the equation:

$$\frac{\text{Stock Sol'n Conc. (µg/g)} \times \text{Wt. of Aliquot (g)}}{\text{50 mL}} = \text{µg/mL} = \text{mg/L}$$

The calibration standards were transferred to 50-mL polypropylene centrifuge tubes with screw-on lids and placed directly in the ICP autosampler.

Sampling Procedure
Samples were taken for analytical verification at 0 hour (before addition of Daphnia) and 48 hours (after removal of Daphnia). Aliquots of the control and test solutions where drawn with 50-mL glass volumetric pipettes. Duplicate 50-mL aliquots were taken directly from the preparation vessels (1 liter volumetric flasks, one flask per loading level) at time = 0 hours. Single 50-mL aliquots were taken from the actual test vessels (two vessels per loading level) at time = 48 hours. The 50-mL aliquots were transferred from the pipettes into 50-mL polypropylene centrifuge tubes with screw-on lids.

Addition of Internal Standard
Germanium (Ge) was added as an internal standard to compensate for instrumental drift. Fifty microliters of a 10,000 µg/mL Germanium solution were added via Unimetrics 100 microliter syringe to the final 50-mL volume of the calibration blank and standards as well as the controls, test solutions, and the diluted QC sample. One hundred microliters of Ge solution was added to the lowest calibration standard (1 mg/L) which was prepared in a 100-mL volumetric. The two spiked QC samples were also prepared so that the concentration of internal standard was the same as for the rest of the samples analyzed, i.e., 10 µg/mL Ge.

Preparation of Spiked Test Solutions
Two spiked test solutions were prepared and analyzed along with the rest of the samples to verify that no matrix interferences were present in the actual test solutions. The amount of product added was calculated to represent the high and low concentrations of the test solutions. Ten microliters of internal standard were added to a 10-mL aliquot of the QC stock solution prior to preparing the spiked test solutions. Using a glass volumetric pipette, 25 mL of each of the control test solutions (no product), also containing 10 µg/mL Ge, were transferred to separate 50-mL polypropylene centrifuge tubes. Approximately 0.35 mL and 3.5 mL of the calibration stock dilution + Ge were added to the 25-mL aliquots of the controls to yield 10 mg/L and 100 mg/L spikes, respectively. The actual amount of QC stock added was weighed using the analytical

Appendix A (cont.)

balance described previously. A portion of each of the spiked test solutions was transferred to individual 15-mL polypropylene centrifuge tubes and placed in the ICP autosampler.

Preparation of Diluted QC Sample

A third QC sample was prepared in a 50-mL volumetric flask in the same fashion as the calibration standards using aerated well water. An aliquot of the QC stock solution was transferred by Rainin pipette and weighed. Internal standard was added after the volumetric flask was filled to the mark. This sample was prepared at approximately 56 mg/L 2-8192 to represent the mid-range of the test solutions. A portion of the diluted QC sample was transferred to an individual 15-mL polypropylene centrifuge tube and placed in the ICP autosampler.

Test Solution Preparation for ICP-OES Analysis

The only preparation procedure required for the 0 (control), 10, 18, and 32, 56, and 100 mg/L test solutions was addition of the internal standard. After addition of internal standard, a portion of each of the controls, test solutions, and QC samples was transferred to individual 15-mL polypropylene centrifuge tubes and placed in the ICP autosampler.

Batch ICP-OES Analysis

The automated analysis mode of the WinLab™ software was used to analyze all the calibration standards, controls, blanks, test solutions, and QC samples from a given day as a batch. An autosampler was used to present all standards and samples to the ICP. The calibration blank and standards were specified in the analysis method and analyzed prior to analysis of the matrix blanks, controls, test solutions, and QC samples. Hard copies of the sequences for both the 0- and 48-hour sample batches are included with the raw data in the study file. Samples were run in the same order they appeared in the sequence. Matrix blanks (aerated well water + Ge) were included to verify that sample carry-over was not impacting Si measurements. The matrix blanks, controls, test solutions, and QC samples were analyzed in triplicate. Including analysis of the calibration blank and standards, ICP analysis of the samples from each day took about 3.5 hours. In order to reduce any potential time-related measurement bias, the replicate analyses of the controls, test solutions, and spikes were performed in subgroups. All the first replicates were analyzed together as were the second and third replicate sets.

ICP-OES Hardware and Conditions

The following hardware and settings were used to generate all the product concentration data for this study.

Instrument:	Perkin Elmer Optima 3100XL ICP-OES
Autosampler:	Perkin Elmer AS-91 (s/n 3675)
Wavelengths:	265.118 nm (Ge)
	251.611 nm (Si)
	288.158 nm (Si)
Nebulizer:	glass concentric
Spray chamber:	glass cyclonic
Plasma Power:	1350 W
Sample flow rate:	1.0 mL/min
Plasma gas flow:	15 L/min
Integration Time:	Auto; 1 s min, 20 s max
Auxiliary gas flow:	0.5 L/min
Nebulizer gas flow:	0.70 L/min

Appendix A (cont.)

Data Collection and Method of Analysis
Processing of raw intensities measured at specific wavelengths was performed using Perkin-Elmer's ICP WinLab™ software, version 1.42, run on a Pentium personal computer under Windows 95. Although data was collected for Si at two of the most prominent emission lines, only the 251.611 nm data was tabulated. The analysis method used to generate all the concentration data included in this memo used internal standardization with linear calibration forced through the origin. All of the raw data for the calibration blank and standards, matrix blanks, test solutions, and QC samples are included in the study file. A printout of the method used each day is included in the study file.

Linearity and Range of Calibration
The calibration was linear over the range of product concentrations evaluated, resulting in a correlation coefficient of 0.9998 with a slope of 3394 cps per mg/L for 0-hour and a correlation coefficient of 0.9999 with a slope of 3072 cps per mg/L for 48-hour analyses. Signal intensities of the calibration blank and standards as well as a calibration summary for each day of analysis are included in the raw data.

Blank Correction
The ICP method measures elemental silicon and is not specific for Therefore, any background Si or Si-containing contamination in the samples will be expressed as product concentration by the ICP software. The well water used for this study contains between 5 mg/L and 10 mg/L native silicon. The calibration blank (aerated well water + Ge) was used to blank correct all the raw data collected during this study. The product concentrations reported for the matrix blanks, test controls, test solutions, and QC samples were calculated by the WinLab™ software after subtraction of the background Si present in the well water. The test controls (Daphnia, 0 mg/L product) were not used to further blank correct the data for the test solutions.

Repeatability
Within-run precision (or repeatability) of the ICP measurements was determined by analyzing all samples in triplicate. The relative standard deviation (RSD) for each set of triplicate measurements was calculated and is included in Tables IV and V at the end of this report. Intermediate precision (between run) was determined by comparing the day-to-day percent recoveries for the spiked test solutions. Spike recoveries measured at 0 and 48 hours are summarized at the bottom of Table III at the end of this report.

Accuracy
The accuracy of the ICP measurements was verified by analysis of spiked QC samples. The following equation was used to calculate spike recoveries.

$$\%\ \text{Recovery} = [(C_{spiked})(V_{spiked}) - (C_{unspiked})(V_{unspiked})] \div \text{mass of test article spiked } (\mu g) \times 100$$

where,
C_{spiked} = mean measured concentration of spiked sample (µg/mL)
V_{spiked} = volume of spiked sample (mL)
$C_{unspiked}$ = mean measured concentration of unspiked sample (µg/mL)
$V_{unspiked}$ = volume of unspiked sample (mL)

Appendix A (cont.)

The recoveries for the diluted QC samples were calculated as shown below.

% Recovery = (mean measured concentration ÷ theoretical concentration)100

The recoveries for the spiked and diluted QC samples are summarized in Table III at the end of this report. The spike recovery calculations by Excel in Table I were all verified using a hand calculator.

Limits of Detection and Quantitation

The method detection limit (MDL) was estimated using the standard deviation of the signal acquired from triplicate analysis of the matrix blanks (aerated well water). Using the test substance calibration curve, the 3σ detection limit was calculated in terms of test substance concentration using the following equation, $MDL = (3)(\sigma_{Blank})/(m)$, where m is the slope of the test substance calibration curve. The resulting MDL is thus expressed as mg/L product and can be compared directly to the test solution and QC sample measurements. The 3σ detection limit for the matrix blank each day of analysis is included in Tables IV and V at the end of this report.

The method quantitation limit (MQL) was also estimated using the standard deviation of the signal acquired from triplicate analysis of the matrix blanks (aerated well water). Using the test substance calibration curve, the 10σ quantitation limit was calculated in terms of test substance concentration using the following equation, $MQL = (10)(\sigma_{Blank})/(m)$, where m is the slope of the test substance calibration curve. The resulting MQL is thus expressed as mg/L product and can be compared directly to the test solution and QC sample measurements. The 10σ quantitation limit for the matrix blank each day of analysis is included in Tables IV and V at the end of this report.

Documentation

Excel for Windows 98, version 9.0.2720, was used to create Tables III, IV, and V at the end of this report. All transcribed data were verified 100%. Twenty percent of the calculations in the spreadsheets were verified using a hand calculator. All of the original ICP-OES reports are located in the study file. Details of calibration blank and standard preparation as well as sampling and preparation of the test controls, test solutions, matrix blanks, and QC samples are recorded in pages 7 through 14. Copies of these notebook pages are included in the study file along with the rest of the analytical data.

Summary of Results

All the acceptance criteria for determination of test substance loading and stability listed in Annex A of the protocol were met for the test solutions analyzed at 0 and 48 hours.

Criteria	Calculation	Range of Acceptance	Results
stability	(conc. @ 48 hrs ÷ conc. @ 0 hrs)100	80 - 120%	92 - 104%
repeatability	(std. dev. ÷ mean measurement)100	≤ 10%	0.079 – 3.5%
accuracy	QC sample analysis (see accuracy section)	85 - 115%	89 - 101%
sensitivity	MDL (see MDL and MQL section)	≤ 10% of lowest test conc.	5 - 7% of lowest conc.

Table II

Appendix A (cont.)

The average percent of product remaining at each test concentration is summarized in Table I. The percent of product remaining for each test vessel is listed in Table III at the end of this report. The percent of product remaining after 48 hours ranged from 92% (10 mg/L, vessel #3) to 104% (18 mg/L, vessel #5). Summaries of all the individual measurements (in triplicate) for 0 and 48 hours are included in Tables IV and V, respectively, at the end of this report. Based on analysis of QC samples, the product concentrations measured during this study were estimated to be accurate to within 11% relative.

Within-run precision (or repeatability) of the test solution measurements, calculated from triplicate measurements and expressed as relative standard deviation (RSD), ranged from 0.079% to 3.5% for the 0-hour measurements and 0.082% to 1.9% for the 48-hour measurements. Comparing the day-to-day spike recoveries, the intermediate precision was estimated to be ≤ 8% relative, based on the recoveries of the 10 mg/L spiked samples. The 10 mg/L spikes were used for this estimation because they had the widest range of recovery (96% to 89%) at 0 and 48 hours.

The method detection limit (MDL) for each batch analysis was estimated by calculating the standard deviation of the matrix blank measurements. The MDLs calculated from the blanks were compared to the concentrations measured for the lowest product loading run on the same day. At both 0 and 48 hours, the 3σ detection limit based on the mean of the matrix blank were all less than 10% of the lowest test concentration measured.

References

1. , "Non-Regulated Study: Development and Validation of an ICP-OES Method to Quantify Test Concentrations of n Aquatic Media", Study TIS report June 27, 2000.

Study # : **Percent of** **Remaining in Test Vessels at 48 Hrs and QC Recoveries**

(Si Measured at 251.611 nm)

Test System Loading		0-Hr Average* mg/L Product per Vessel	48 Hr Average* mg/L Product per Vessel	Percent of 2-8192 @ 48 Hr Relative to Conc. Measured at 0-Hr
10 mg/L product	Vessel #3	10.75	9.868	91.8%
	Vessel #4	9.388	9.116	97.1%
16 mg/L product	Vessel #5	16.95	16.55	104%
	Vessel #6	16.37	15.47	94.5%
32 mg/L product	Vessel #7	32.47	30.82	94.9%
	Vessel #8	32.47	30.10	92.7%
56 mg/L product	Vessel #9	56.88	53.84	94.7%
	Vessel #10	56.12	53.81	95.9%
100 mg/L product	Vessel #11	101.7	94.44	92.9%
	Vessel #12	102.2	94.94	92.9%

Unspiked & Spiked Results		Aver.* 0-Hr 2-8192 (mg/L)	Aver.* 48 Hr 2-8192 (mg/L)
unspiked	Sample/Vessel #1	-0.424	2.209
unspiked	Sample/Vessel #2	-0.875	1.266
~ 10 mg/L product spiked	Sample/Vessel #1	9.160	11.24
~ 100 mg/L product spiked	Sample/Vessel #2	87.51	97.40

Mass of 2-8192 Spiked	0-Hr Stock Conc. (ug/g 2-8192)	0-Hr Spike Wt. (g)	Mass of 0-Hr Spike (ug)	48 Hr Stock Conc. (ug/g 2-8192)	48 Hr Spike Wt. (g)	Mass of 48 Hr Spike (ug)
Vessel #1	738.0	0.3411	251.7	791.0	0.3245	256.7
Vessel #2	738.0	3.3682	2484	791.0	3.5509	2809

		0-Hr	48 Hr
Spike Recoveries	~ 10 mg/L product	86%	89%
	~ 100 mg/L product	101%	98%

Diluted QC Recovery		0-Hr	48 Hr
Aver.* Measured mg/L 2-8192		57.17	55.18
Theoretical mg/L 2-8192		56.47	56.24
Recovery	~ 56 mg/L product	101%	98%

* Each solution was analyzed in triplicate.

Table III

Study #	0-Hour ICP Results for	(duplicate samples removed from preparation vessels)							
251.611nm									
Sample Description		Rep. #1 (mg/L product)	Rep. #2 (mg/L product)	Rep. #3 (mg/L product)	Std. Dev. per Sample (mg/L product, n = 3)	Mean Measurement (mg/L product, n = 3)	RSD per sample (mg/L product)	LOD Mean + (3xs.d.) (mg/L product)	LOQ Mean + (10xs.d.) (mg/L product)
Matrix Blank (aerated well water)	stored on bench	0.053	0.025	0.262	0.13	0.11	110%	0.50	1.4
		Sample Rep. #1 (mg/L product)	Sample Rep. #2 (mg/L product)	Sample Rep. #3 (mg/L product)	Average mg/L Product per Sample (n = 3)	Std. Dev. per Sample (n = 3) (mg/L product)	RSD per Sample (mg/L product)		
Test Control (0 mg/L product)	Sample #1	-0.446	-0.428	-0.397	-0.424	0.0246	5.9%		
	Sample #2	-0.910	-0.873	-0.843	-0.875	0.0336	3.8%		
10 mg/L Product Test	Sample #1	10.34	10.83	11.07	10.75	0.3721	3.5%		
	Sample #2	9.169	9.618	9.378	9.388	0.2247	2.4%		
16 mg/L Product Test	Sample #1	15.76	16.04	16.06	15.95	0.1677	1.1%		
	Sample #2	16.36	16.37	16.39	16.37	0.01528	0.093%		
32 mg/L Product Test	Sample #1	32.32	32.49	32.61	32.47	0.1457	0.45%		
	Sample #2	32.36	32.43	32.62	32.47	0.1345	0.41%		
56 mg/L Product Test	Sample #1	56.88	56.93	56.84	56.88	0.04509	0.079%		
	Sample #2	56.14	56.15	56.06	56.12	0.04933	0.088%		
100 mg/L Product Test	Sample #1	101.7	101.9	101.4	101.7	0.2517	0.25%		
	Sample #2	102.3	102.1	102.1	102.2	0.1155	0.11%		
QC Samples									
~ 56 mg/L Diluted QC Smpl	Made w/ aerated well water	57.01	57.17	57.32	57.17	0.1550	0.27%		
~ 10 mg/L product spike	Made w/ Test Cntrl #1	8.831	9.302	9.348	9.160	0.2861	3.1%		
~ 100 mg/L product spike	Made w/ Test Cntrl #2	87.60	87.58	87.35	87.51	0.1389	0.16%		

Table IV

Study #	48-Hour ICP Results for	(duplicate test systems, Daphnia removed prior to analysis)							
251.611nm									
Sample Description		Rep. #1 (mg/L product)	Rep. #2 (mg/L product)	Rep. #3 (mg/L product)	Std. Dev. per Sample (mg/L product, n = 3)	Mean Measurement (mg/L product, n = 3)	RSD per sample (mg/L product)	LOD Mean + (3xs.d.) (mg/L product)	LOQ Mean + (10xs.d.) (mg/L product)
Matrix Blank (aerated well water)	stored on bench	0.213	0.316	0.474	0.131	0.334	39%	0.729	1.65
		Sample Rep. #1 (mg/L product)	Sample Rep. #2 (mg/L product)	Sample Rep. #3 (mg/L product)	Average mg/L Product per Sample (n = 3)	Std. Dev. per Sample (n = 3) (mg/L product)	RSD per Sample (mg/L product)		
Test Controls (0 mg/L product)	Vessel #1	2.190	2.291	2.145	2.209	0.07477	3.4%		
	Vessel #2	1.377	1.223	1.197	1.266	0.09729	7.7%		
10 mg/L Product Test	Vessel # 3	9.688	9.929	9.987	9.868	0.1585	1.6%		
	Vessel # 4	8.913	9.212	9.223	9.116	0.1759	1.9%		
18 mg/L Product Test	Vessel # 5	16.53	16.53	16.60	16.55	0.04041	0.24%		
	Vessel # 6	15.38	15.47	15.57	15.47	0.09504	0.61%		
32 mg/L Product Test	Vessel # 7	30.80	30.82	30.85	30.82	0.02517	0.082%		
	Vessel # 8	29.99	30.18	30.13	30.10	0.09849	0.33%		
56 mg/L Product Test	Vessel # 9	53.87	53.99	53.86	53.84	0.1609	0.30%		
	Vessel # 10	53.58	53.87	53.95	53.81	0.2066	0.38%		
100 mg/L Product Test	Vessel # 11	94.10	94.74	94.48	94.44	0.3219	0.34%		
	Vessel # 12	94.69	95.06	95.07	94.94	0.2166	0.23%		
QC Samples									
~ 56 mg/L Diluted QC Smpl	Made w/ aerated well water	54.85	55.24	55.46	55.18	0.3089	0.56%		
~ 10 mg/L product spike	Made w/ Test Cntrl #1	10.88	11.31	11.54	11.24	0.3350	3.0%		
~ 100 mg/L product spike	Made w/ Test Cntrl #2	97.40	97.16	97.84	97.40	0.2400	0.25%		

Table V

Appendix B

Study

MEMORANDUM

To:

From:

Subject: Results of the analysis of the data from study

Summary

The purpose of this study was to determine the LC_{50} of in an aquatic environment using the survival of *Daphnia magna* after 24 hours and 48 hours of exposure as the endpoint. The LC_{50} at 24 hours was 62.91 ppm with a 95% fiducial interval of (52.12, 78.07). The LC_{50} at 48 hours was 34.02 ppm with a 95% fiducial interval of (29.65,39.13).

Introduction

The purpose of this study was to determine the LC_{50} of in an aquatic environment using the survival of *Daphnia magna* after 24 hours and 48 hours of exposure as the endpoint. Five concentrations of of (10, 18, 32, 56, and 100 ppm) were tested against untreated controls.

Methods

Data analysis was carried out using SAS v. 8. A probit analysis of the number of deaths in each group at the given time interval was used to determine the LC_{50}. The initial analysis of the data indicated that the probit model was appropriate for the 24 hour data but not the 48 hour data. In the 48 hour data, there was one death in the 10 ppm group but no deaths in the 18 ppm group. After reviewing the dose response at 24 and 48 hours, the study director determined that the one death at 10 ppm was not test article related. I agreed with this assessment and the LC_{50} for the 48 hour time period was calculated using a data set without the 10 ppm data.

Results

The LC_{50} at 24 hours was 62.91 ppm with a 95% fiducial interval of (52.12, 78.07). The LC_{50} at 48 hours was 34.02 ppm with a 95% fiducial interval of (29.65,39.13).

Documentation

A copy of the SAS® log and SAS® output for this analysis is maintained in the study file.

Signature _ **Date** 10/6/2003